

Tsuneo FUNAMOTO* & Ryuso TANAKA**: **Karyomorphological studies on the genus *Chrysosplenium* in Japan (1) Four species in section *Nephrophyloides***

船本常男*・田中隆莊**: 日本産ネコノメソウ属の核形態学的研究 (1)
ヤマネコノメソウ節の4種

The genus *Chrysosplenium*, with wide distribution in arctic to temperate regions of the world, consists of approximately 55 species. About forty-six species are concentrated in temperate Asia, mainly in China and Japan; 14 occur in Japan (Hara 1957). Although chromosome studies of seven species have been made with the documented chromosome numbers of $2n=22$, 24 and ca. 72 by various authors (e.g., Matsuura & Sutô 1935, Hara & Kurosawa 1963, Kurosawa 1977, 1983), karyomorphological analysis, however, has been poorly described in most standard references.

Materials and methods The materials collected in various places and cultivated in pots are listed in Tab. 1. Taxonomical treatment followed Hara (1957). For observation of somatic chromosomes, root tips were pretreated with 0.002 M 8-hydroxyquinoline for 4 hrs at about 20°C, and fixed in 45% acetic acid for 10 min at about 2°C. The fixed root tips were macerated in a mixture of 1N HCl and 45% acetic acid (1:1) for about 20 sec at 60°C, stained and squashed in 2% aceto-orcein. Grouping of chromosomes by position of centromere followed Levan et al. (1964).

Observations The chromosome numbers of the four species were all $2n=24$ (Tab. 1). Morphology of chromosomes at resting and mitotic metaphase are described as follows:

1) *Chrysosplenium japonicum* (Maxim.) Makino, $2n=24$ (Fig. 1A, B, Fig. 2A).

The nucleus was about $6.6 \mu\text{m}$ in diameter and formed chromatin threads, and many chromomeric granules and chromocentral blocks were scattered in the

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Tab. 1. Chromosome numbers and localities of four species of *Chrysosplenium* investigated.

Species	Chromosome number (2n)	Locality
<i>C. japonicum</i>	24	Okayama Prefecture, Maniwa-gun, Katsuyama-cho, Kanpano-taki
	24	Shimane Prefecture, Ochi-gun, Sakurae-cho, Ryuzuno-taki
	24	Yamaguchi Prefecture, Kuga-gun, Nishiki-cho, Mt. Jakuchi
	24	Kochi Prefecture, Tosa-gun, Tosa-cho, Sameura
<i>C. tosaense</i>	24	Saitama Prefecture, Hannou city, Mt. Ohkura
	24	Hiroshima Prefecture, Saeki-gun, Yuki-cho, Mt. Akezu
<i>C. alternifolium</i> var. <i>sibiricum</i>	24	Hokkaido Province, Sorachi, Uryu-gun, Shokanbetsu
<i>C. flagelliferum</i>	24	Nagano Prefecture, Kamiminochi-gun, Togakushi-mura

whole region of the nucleus at the resting stage. Approximately six chromocentral blocks per nucleus were either elliptical or spherical, and varied in size from 0.5-0.7 μm along the major axis (Fig. 1A). Morphology of the resting chromosomes is of the simple chromocenter type according to Tanaka (1971, 1980). The somatic chromosome number of $2n=24$ supported the previous report of Kurosawa (1977). From the longest chromosome of 1.1 μm to the shortest chromosome of 0.6 μm , a gradual decrease in size was observed in the chromosome complement which was composed of the metacentric and submetacentric chromosomes. Thus, it was categorized as a symmetric karyotype according to arm ratio. A satellite was observed on the short arm of the smallest chromosome (Fig. 2A).

2) *Chrysosplenium tosaense* Makino, $2n=24$ (Fig. 1C, D, Fig. 2B).

The nucleus was about 6.4 μm in diameter. The morphological characteristics of the resting chromosomes were similar to those of *C. japonicum* described above. Approximately ten heteropycnotic bodies per nucleus varied in size from 0.4-0.6 μm along the major axis (Fig. 1C). The chromosome number

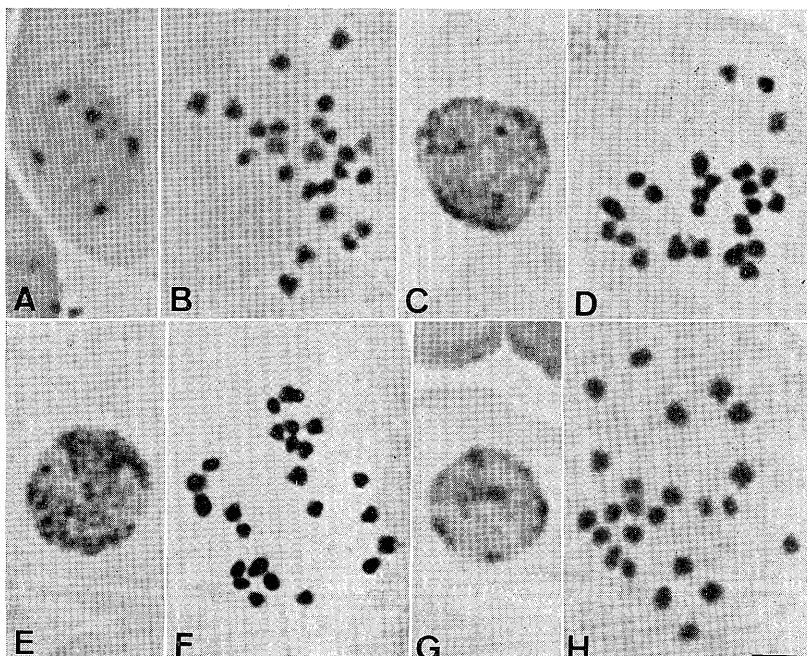


Fig. 1. Photomicrographs of somatic chromosomes of four species of *Chrysosplenium* ($2n=24$).

A, C, E and G: resting stage. B, D, F and H: mitotic metaphase chromosomes. A and B: *C. japonicum*. C and D: *C. tosaense*. E and F: *C. alternifolium* var. *sibiricum*. G. and H: *C. flagelliferum*. Bar represents $2 \mu\text{m}$.

of this species was counted here for the first time. From the longest chromosome of $1.4 \mu\text{m}$ to the shortest chromosome of $0.7 \mu\text{m}$, a gradual decrease in chromosome length was observed in the chromosome complement. The first to fourth chromosomes were classified as submedian, and the other 5th to 24th chromosomes were classified as median. No satellited chromosome was observed.

3) *Chrysosplenium alternifolium* L. var. *sibiricum* Seringe ex DC., $2n=24$ (Fig. 1E, F, Fig. 2C).

The resting nucleus was about $5.8 \mu\text{m}$ in diameter, and contained about 13 heteropycnotic bodies in the whole region, which varied in size from 0.4 – $0.6 \mu\text{m}$ along the major axis (Fig. 1E). The chromosome number supported the previous report of Kurosawa (1983). From the longest chromosome of $1.2 \mu\text{m}$ to the shortest chromosome of $0.7 \mu\text{m}$, a gradual decrease in chromosome length was

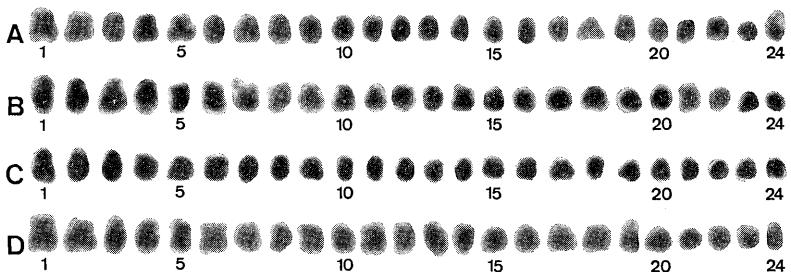


Fig. 2. Mitotic metaphase chromosomes of four species of *Chrysosplenium*. A: *C. japonicum*. B: *C. tosaense*. C: *C. alternifolium* var. *sibiricum*. D: *C. flagelliferum*. Bar represents 2 μ m.

observed in the chromosome complement composed of median and submedian chromosomes. No satellites chromosome was observed.

4) *Chrysosplenium flagelliferum* Fr. Schmidt, $2n=24$ (Fig. 1G, H, Fig. 2D).

The resting nucleus was about 5.6 μ m in diameter, and contained about seven heteropycnotic bodies in the whole region, which varied in size from 0.5–0.6 μ m along the major axis (Fig. 1G). The chromosome number of $2n=24$ supported the previous report of Matsuura & Sutô (1935). From the longest chromosome of 1.3 μ m to the shortest chromosome of 0.8 μ m, a gradual decrease in chromosome length was observed in the chromosome complement composed of metacentric and submetacentric chromosomes. No satellites chromosome was observed.

Discussion The somatic chromosome number of $2n=24$ of *C. japonicum*, *C. alternifolium* var. *sibiricum* *C. flagelliferum* confirmed the previous counts, while that of *C. tosaense* was reported here for the first time. Although *C. japonicum* showed chromocentral blocks more clearly than the other three species, the four species showed very similar resting chromosomes; the morphological feature was of the simple chromocenter types categorized by Tanaka (1971, 1980).

According to Hara (1957), the four species are taxonomically clearly distinguished from each other. Cytological features showed the four species had similar karyotypes, except *C. japonicum* had certain, clear chromocentral blocks at resting stage. Thus, it is concluded that the four species could be karyomorphologically included in the same group which compatible with the taxonomic treatment of *Chrysosplenium* by Hara (1957).

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References

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日本産ネコノメソウ属ヤマネコノメソウ節の4種、ヤマネコノメソウ、タチネコノメソウ、エゾネコノメソウ、ツルネコノメソウの核形態的研究を行なった。体細胞染色体数は4種ともに $2n=24$ であった。本研究によって初めて染色体数が算定された種はタチネコノメソウ (*C. tosaense*) である。なお、他の3種については従来の報告と一致した。静止期染色体の形態は4種ともに単純染色中央粒型であったが、ヤマネコノメソウ (*C. japonicum*) の静止期染色体の形態は他の3種より染色中央粒の輪郭が明瞭であった。分裂期中期染色体は4種ともに小形で長さが勾配的、動原体は median あるいは submedian 型であった。種の間の核型的区別は困難である。日本産ヤマネコノメソウ節の4種は $x=12$ を基本数とし、核形態的にはよくまとまった節といえる。